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DETERMINATION OF CARBOXYHEMOGLOBIN IN DECOMPOSED BODIES

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FOREWORD

The investigations described in this report were performed during the period from March 1962 to March 1963. Captain Abel M. Dominguez, USAF, MSC, was the principal investigator, and the research was conducted at the Armed Forces Institute of Pathology, Washington 25, D. C., and Ramey Air Force Base, Puerto Rico, under MIPR (33-657)-2-RD-175, Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630205, "Toxic Hazards Evaluation" with the Biomedical Laboratory, 6570th Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson AFB, Ohio. Captain Roman L. Patrick, Toxic Hazards Branch, Physiology Division, Biomedical Laboratory, was contract monitor for the 6570th Aerospace Medical Research Laboratories.

The authors gratefully acknowledge the valuable suggestions and assistance of Dr. Leo R. Goldbaum of the Armed Forces Institute of Pathology in accomplishing the analysis for carbon monoxide, and the support of Major William H. Grau, Jr., USAF, VC, at Ramey Air Force Base, Puerto Rico, who assisted in logistics planning and support.

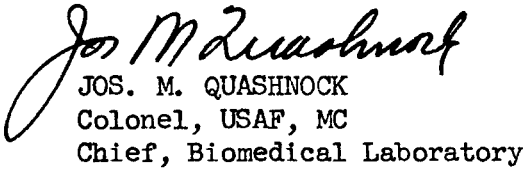
Animal experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

ABSTRACT

This study is concerned with the interpretation and the significance of carbon monoxide findings in cases associated with decomposition. The evidence presented indicates that the percentage of carboxyhemoglobin saturation value is not markedly altered during postmortem decomposition when: 1) the specimens are properly preserved (i.e., upon collection the specimens are rapidly frozen and maintained in a frozen state until examined for carbon monoxide), and 2) the blood extracted from tissue is examined as soon as possible for the presence of carbon monoxide. Under the conditions studied, it appears possible to obtain carbon monoxide values, utilizing blood extracted from tissue, in the presence of postmortem decomposition that are similar to the antemortem levels. For example, within the 4% to 12% carboxyhemoglobin-saturation range covered in this study, there was neither a marked increase nor decrease in final percentage of carboxyhemoglobin following decomposition. Nevertheless, unreliable carboxyhemoglobin saturation values may occur, and the possible contributory factors responsible for a spurious increase in carboxyhemoglobin values are discussed along with corrective measures. A gas-solid chromatographic procedure for determining carbon monoxide using blood or tissue is presented. This procedure employs a Van Slyke apparatus for liberating gases from biological specimens modified for introducing released gas into the gas chromatograph.

PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.



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DETERMINATION OF CARBOXYHEMOGLOBIN IN DECOMPOSED BODIES

INTRODUCTION

1. Statement of the Problem.

The data here reported relate to the interpretation and the significance of those positive carbon monoxide findings that are associated with decomposition of the biologic test specimen.

2. Background Information:

a. Testing for the presence of carbon monoxide is an integral part of the toxicologic examination of blood and of hemoglobin derived from the solid tissues of victims of fatal aerospace accidents. The postmortem analysis of tissues for the presence of carbon monoxide is likewise essential to the medical investigation of various other fatalities, notably those associated with fire.

b. The use of gas chromatography (ref. 4) has enhanced the accuracy and specificity of the toxicologic analysis for carbon monoxide and has made possible the analysis of test specimens consisting of dilute aqueous extracts of the hemoglobin contained in small amounts of the postmortem tissues.

c. In aircraft accident cases, the interpretation of the significance of carbon monoxide findings derived from the toxicologic examinations has become facilitated by the emergence of two comprehensive generalizations (ref. 3): (1) carboxyhemoglobin saturation values of less than 10% saturation must be interpreted with caution, since values of less than this degree are consistent with smoking, and (2) carboxyhemoglobin saturation values of greater than 10% saturation indicate that the victim was alive at the time of exposure to carbon monoxide (e.g., in fumes from fire). Single and together, these two generalizations focus attention upon the 10% carboxyhemoglobin saturation value as a point of reference for the practical interpretation of the significance of postmortem carbon monoxide findings.

d. Residual problems concerned with the interpretation of postmortem carbon monoxide findings do persist. To the medical investigator the more troublesome of the latter problems are those concerned with the validity of carbon monoxide findings derived from the analysis of decomposed specimens. On the one hand, one might ask whether carbon monoxide production is or may be a concomitant of decomposition. On the other hand, one is equally concerned with the question of whether carbon monoxide present in the body at the time of death decreases or disappears, if subsequently exposed

to the cumulative process of tissue decomposition. In our experience these problems have manifested themselves infrequently but are of critical importance to the medical investigator; e.g., the interpretation of elevated carbon monoxide findings in victims of fatal over-water accidents in which delays in the recovery of the bodies were associated with tissue decomposition.

e. The present study was designed principally to investigate the first of the two problems described in the preceding paragraph -- the production of carbon monoxide as a concomitant of tissue decomposition. Intact animals were immersed in sea water (offshore waters of Puerto Rico) until an advanced state of decomposition was present. Carbon monoxide determinations were then performed on the blood and on aqueous extracts of selected organs of the separate animals.

METHODS AND MATERIALS

Dogs weighing 9.1 to 13.6 Kgm. and ranging in age from 2 to 4 years were used in this study. The animals were immunized against canine distemper, canine hepatitis, rabies, and, in addition, they were dewormed and maintained in quarantine for approximately 1 month prior to use.

The animals were divided into two groups. One group, consisting of eight animals, was exposed to a mixture of 1% carbon monoxide in air in order to achieve a relatively low level of blood carbon monoxide prior to submersion in sea water. The second group, with seven animals, was utilized as the control group, and these animals were not exposed to carbon monoxide prior to water submersion. Blood specimens were collected in heparinized syringes from both groups of animals and transferred to tubes containing sodium fluoride. The contents of the tubes were then rapidly frozen and maintained in this state until examined for carbon monoxide at the Armed Forces Institute of Pathology (AFIP).

Following collection of the blood specimens for quantitative determination of carbon monoxide by use of gas-solid chromatography, the test subjects were submerged in marine plywood containers (Fig. 1) constructed specifically for this study. In the carbon monoxide-treated group, the elapsed time between carbon monoxide exposure, removal of blood, and submersion of the subjects in sea water did not exceed 10 minutes. The containers were anchored 25 to 30 feet below the surface, in a selected area where water depth was 50 feet. The temperature of the water in this area during the period of the experiment was 29.5°C.

Collection of Samples:

After 2 days the containers were hoisted to the surface, the animals examined to determine their condition, and then resubmerged to the same water depth. On the fourth day the animals were again brought to the surface, examined, and dissected at the experimental site to obtain tissue specimens.

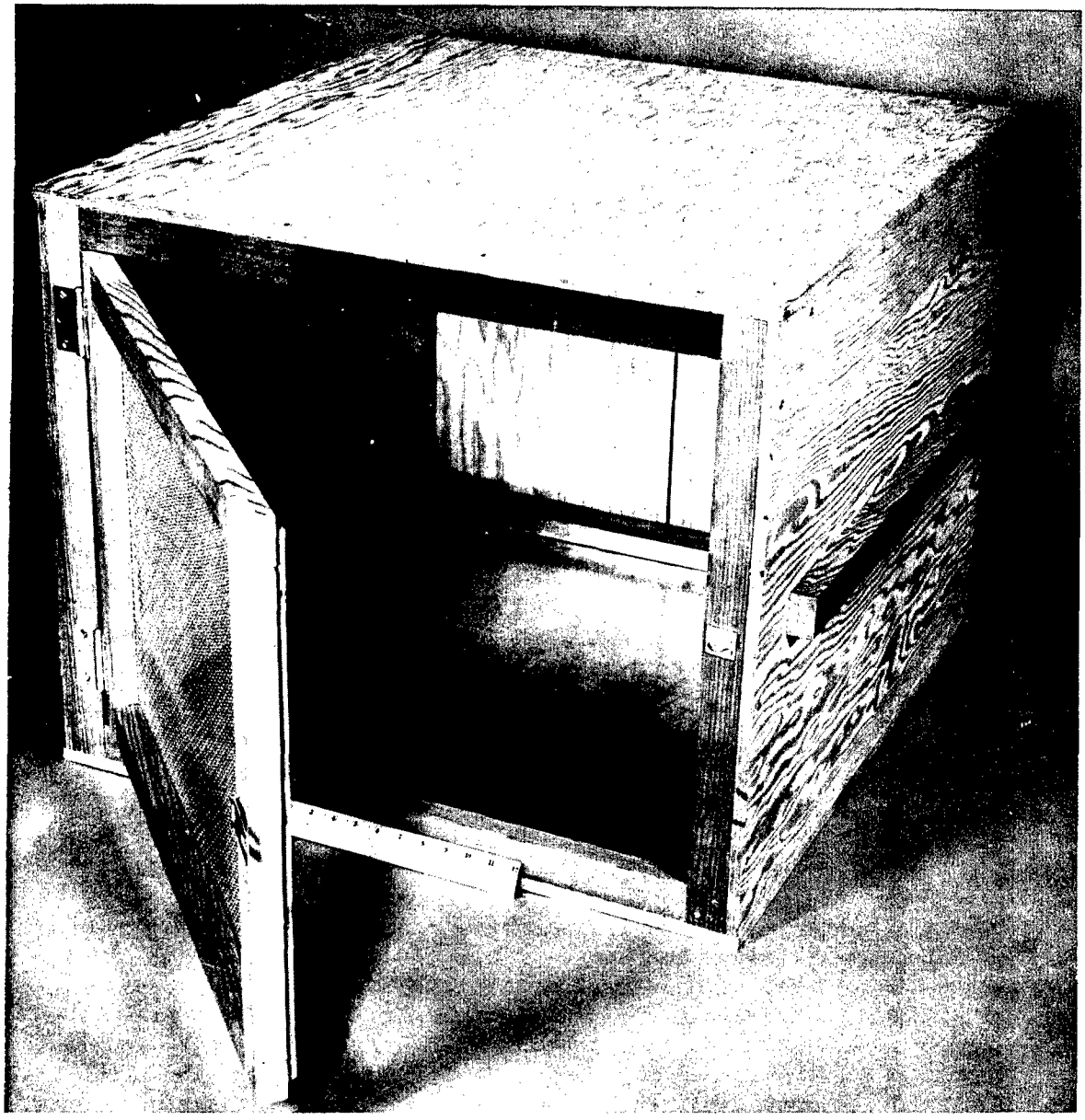

Figure 1. Specially constructed water-resistant box (24" x 30" x 30") used for shipping and submersion studies. It was made of marine plywood with redwood frame, brass screws, and galvanized steel tray and wire mesh.

The desired level of postmortem decomposition was confirmed by the findings of slippage of the hair, deterioration of the eyes, discoloration of the skin, breakdown products of extravasated subcutaneous blood, the softness and the sponginess of the tissues on palpation, and the characteristic odor. The following biologic samples were collected for carbon monoxide evaluation: liver, kidney, the lungs and heart in toto, and any sanguineous fluid present in the thoracic cavity. These specimens were rapidly removed and inserted in plastic bags, and the latter were placed in special insulated shipping containers filled with dry ice. To insure that no thawing would take place, the frozen specimens, in the shipping containers, were taken directly to the AFIP by the investigators. At the AFIP, the frozen specimens were stored at -15°C.

Preparation of Sample:

In selecting the specimen for carbon monoxide evaluation, the material collected from the animals was handled in the same manner as for a routine investigation of an aircraft accident. For example, in routine aircraft-accident investigations tissue is selected, on the basis of past experience, that will give a high hemoglobin concentration on aqueous extraction, and it is carefully examined for carbon monoxide. The experimental material was selected from the dogs in a similar manner for this study. A specimen of heart and lung tissue, frozen in toto, proved to be the most satisfactory material for analysis. The specimen was divided in two portions.

a. One portion was maintained in a frozen condition until prepared for study. When the blood was removed from this tissue with water, the aqueous extract was examined for the presence of carbon monoxide without delay.

b. The second portion was transferred to plastic bags and handled in the following manner: Preliminary studies were initiated to explore the effect on the final percentage of carboxyhemoglobin when a frozen specimen is allowed to thaw and remain in this condition for a period of time. This was an attempt to simulate, to a degree, the situation that might occur during shipment of fresh frozen tissue to the AFIP from aircraft-accident cases. For this study, the plastic bags containing the remaining portion of the heart-lung mixture, not examined in a frozen condition, was allowed to thaw and remain in this state for at least 24 hours, during which time the ambient temperature was about 25°C. Following this treatment, the samples were returned to the freezer and stored until analyzed for carbon monoxide. In preparing the heart and lung tissue for carbon monoxide study, the refrozen sample was placed in a beaker, diced, a small amount of water added, the beaker covered with aluminum foil, and the contents allowed to remain at room temperature until the specimen was no longer frozen. Finally, the materials of the beaker were filtered through gauze into a glass-stoppered cylinder and analyzed in about one hour. A quantity of filtrate amounting to 30 to 50 ml was usually available for study, and the analysis for carbon monoxide was performed on this extract.

The total quantity of hemoglobin in the extract was determined by the maximum amount of carbon monoxide the extract could bind. To establish the carbon monoxide capacity of the extracts from the thawed cases, approximately 5 ml. quantities of the sample were added to two separate 30 ml. syringes. To one of the syringes, a small quantity of sodium hydrosulfite ($Na_2S_2O_4$) was added. The syringes were filled with carbon monoxide gas to saturate both the sodium hydrosulfite-treated and the nontreated extract. Since methemoglobin lacks the capacity to combine with carbon monoxide, sodium hydrosulfite was added to reconvert methemoglobin to reduced hemoglobin. Thus, all the available hemoglobin in the extract was in a form capable of combining with carbon monoxide. The carbon monoxide concentration was determined by the use of gas chromatography.

Determination of Carbon Monoxide:

In this study, the gas-solid chromatographic procedure (ref. 4) developed at the AFIP for the determination of carbon monoxide has been modified for the liberation and introduction of this gas into the gas chromatograph.

1. Preparation of Sample: Blood is diluted 1:10 with distilled water. The dilution of blood yields a more uniform sample and permits identical aliquots to be removed for carbon monoxide content and carbon monoxide capacity. Blood extracted from tissue (e.g., liver, lung, or kidney) may also be utilized. It is advisable to select the specimen containing the most blood. Approximately 10 gm. of tissue will suffice, ordinarily, but it may be necessary to use up to 50 gm. when the tissue contains little blood. The external surface of the tissues should be removed and discarded to preclude contamination. The remaining tissue is diced and distilled water is added to extract the blood from the tissue. The quantity of water depends on the amount of blood in the tissue. Ordinarily, 20 ml. of water produces a satisfactory extract, although the final volume in some instances has been as high as 45 ml. The analysis for carbon monoxide is performed on this extract. Aqueous tissue extracts prepared from decomposed cases should be examined for the presence of carbon monoxide as soon as possible after preparation, because these diluted hemoglobin solutions will deteriorate rapidly, and thus make the results difficult to interpret.

2. Liberation of Carbon Monoxide from Sample: Figures 2 and 3 show a modified Van Slyke apparatus utilized for liberating and introducing the gas sample for analysis. A gas-sampling chamber with a special four-way stopcock (top stopcock) has been attached to the waste tube of the extraction chamber of the Van Slyke apparatus. With the mercury reservoir bulb in the upper position, the extraction chamber and its bore are completely filled with mercury up to the bottom of the intake cup. The middle stopcock (Fig. 2) is closed, and approximately three drops of caprylic alcohol are added to the intake cup. One to 10 ml. of the sample to be analyzed is added to the cup and admitted into the extraction chamber by opening the middle and lower stopcocks after the mercury reservoir bulb is moved to the lower position. Only the sample and about two drops of the caprylic alcohol is allowed to enter the chamber before the middle stopcock is closed.

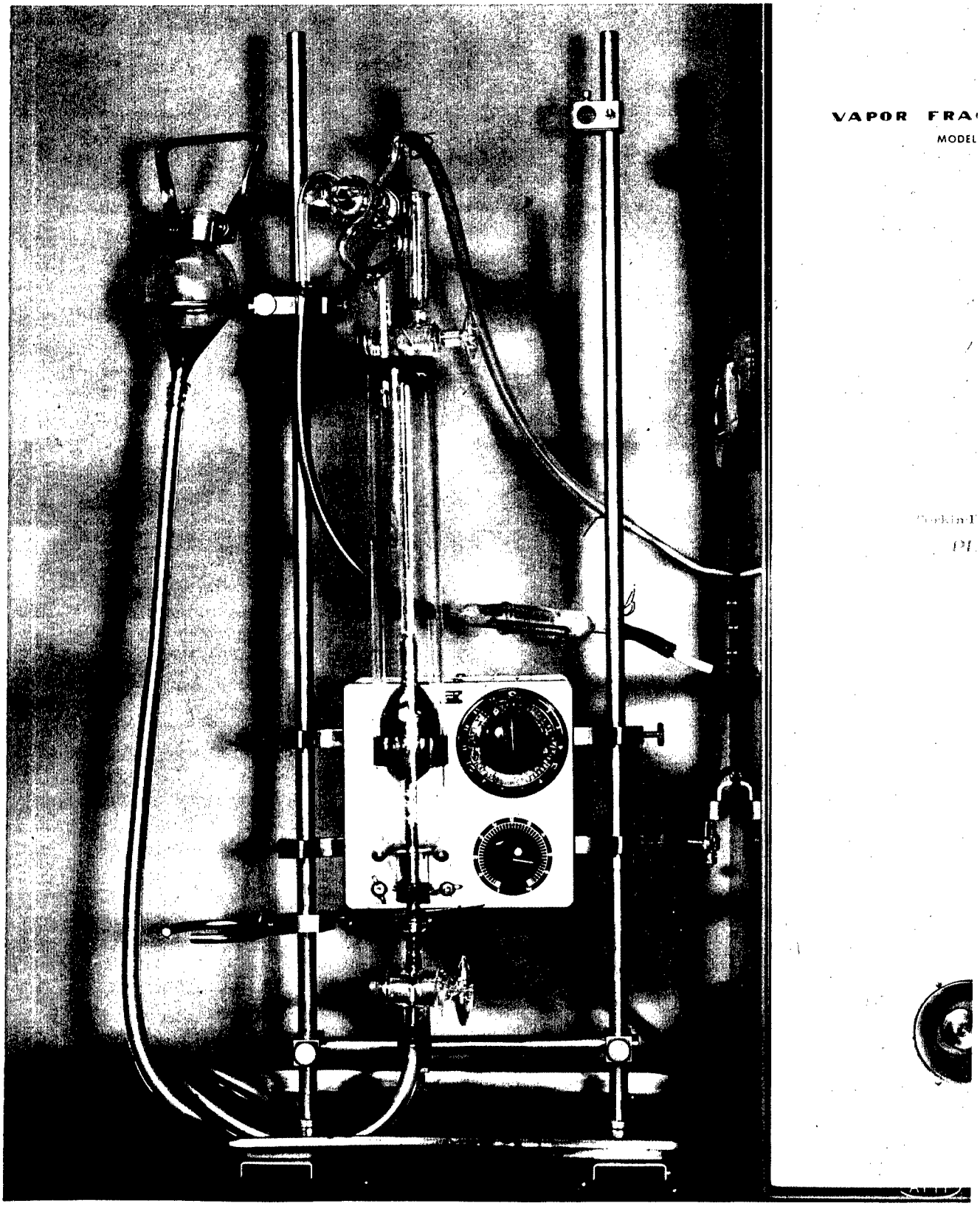

Figure 2. Van Slyke apparatus for liberating carbon monoxide from biologic specimens, modified for introducing released gas into the gas chromatograph.

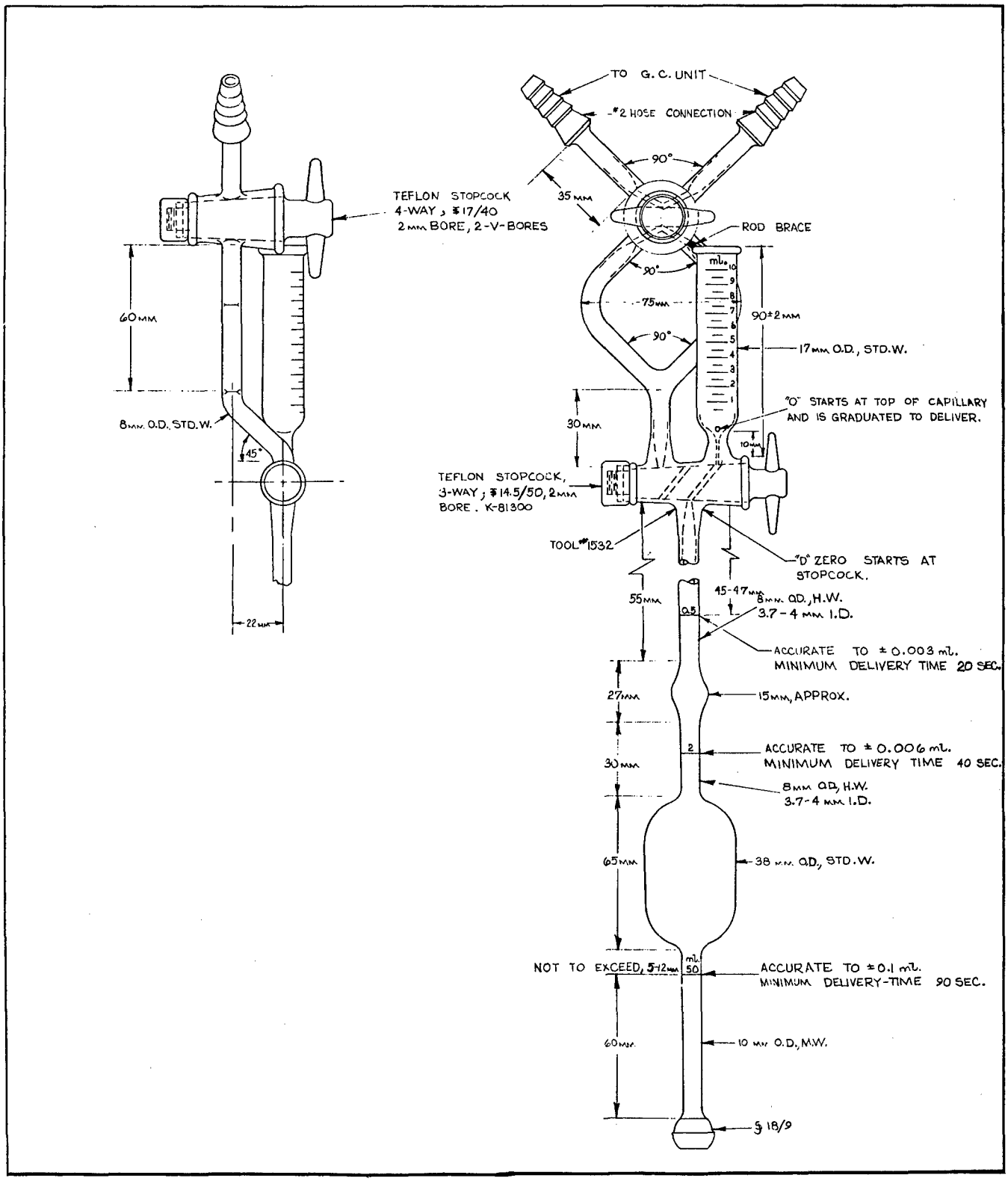

Figure 3. Detailed specifications for the construction of special four-way stopcock and its attachment to Van Slyke apparatus.

In order to liberate carbon monoxide from carboxyhemoglobin, 5 ml. of 85% lactic acid is added to the sample cup and then drawn into the extraction chamber by turning the stopcock as above, taking care not to introduce air. The sample, the lactic acid solution, and mercury within the extraction chamber are lowered to about the mid-level point of the extraction chamber. The oxygen normally present, combined with the acid is sufficient to release the carbon monoxide from carboxyhemoglobin. For ease of handling, preparation, and cleaning of the apparatus, this solution is preferred to the commonly used ferricyanide reagent. The mixture, which is under negative pressure, is agitated for 3 minutes. The mercury reservoir is raised to its upper position (Fig. 2). The extracted gases and mixture in the chamber are run up smoothly, and when the upper meniscus of the solution reaches the bifurcation of the gas-sampling chamber, it is stopped by turning off the middle stopcock. The recorder motor is turned on. The upper stopcock is turned to allow the carrier gas, helium, to sweep the released gases present in the gas-sampling chamber into the gas chromatograph. At this point, and prior to the elution of the gases from the column, the recorder base line is adjusted, if necessary. After carbon monoxide has emerged from the gas chromatograph column and has been recorded, the upper stopcock of the gas-sampling chamber is turned, thus sealing this chamber from the flow of the carrier gas. By the appropriate manipulation of the stopcocks, the solutions are first removed from the neck of the gas-sampling chamber, lowered into the extraction chamber, and then ejected through the intake cup. The system is rinsed with water to clean the apparatus prior to the introduction of the next sample.

3. Measurement of Carbon Monoxide Capacity: The total quantity of hemoglobin in a specimen is determined by the maximum amount of carbon monoxide the blood or extract can bind. To establish the carbon monoxide capacity, usually 5 to 10 ml. of the sample is placed in a 30 ml. syringe and the remainder of the space filled with carbon monxide gas. The syringe is then filled with helium and again shaken for a few minutes. Following this, the gas is completely expelled. This procedure is repeated to remove all the physically dissolved carbon monoxide. A suitable aliquot, depending upon the hemoglobin concentration of the carbon monoxide-saturated sample, is transferred to the Van Slyke apparatus, and the gas is liberated as described above and introduced into the gas chromatograph for analysis.

4. Gas Chromatograph: The gas chromatograph routinely used at the Armed Forces Institute of Pathology for the determination of carbon monoxide is a Perkin-Elmer Vapor Fractometer, Model 154D, employing an 8,000 ohm, thermister-type thermal-conductivity unit as the detector, and a 1-mv Leeds and Northrup Speedomax, Type G Recorder, Model S, 60000 Series. For the separation of carbon monxide from other gases, a 2-m partition column, $\frac{1}{4}$ inch in diameter, packed with Fisher molecular sieve Type 5A, 1/6 inch pellets, is used. When a new column is prepared and inserted into the instrument, it is activated by heating for several hours at about 190°C. For the routine determination for carbon monoxide, the column temperature is maintained at 75°C, and helium is employed as the carrier gas, at a flow rate of 135 ml. per minute. At this temperature and flow rate, carbon monoxide is separated within 5 minutes following the introduction of the sample.

5. Calculation: The percent carboxyhemoglobin in the specimen is derived by comparing the peak height readings of carbon monoxide content and carbon monoxide capacity. The fact that carbon monoxide content and carbon monoxide capacity are determined in an identical manner serves to nullify any inherent errors that may arise in the analysis. The actual carbon monoxide content value is not actually necessary, except in terms of peak height. The amount of carbon monoxide in the gas sample is calculated by measuring the peak height, i.e., the distance from the center of the base of the peak to its maximum height.

If:

A = peak height measurement of unsaturated specimen (CO content),
A_S = peak height measurement of saturated specimen (CO capacity),
V = volume of unsaturated specimen, and
V_S = volume of saturated specimen,

Then: The following formula may be used to establish percent carboxyhemoglobin in a given specimen:

$$\frac{A \times V_S \times 100}{A_S \times V} = \text{Percent carboxyhemoglobin}$$

This method has been employed in over 2,000 aircraft-accident cases for the determination of carbon monoxide in specimens consisting of blood and blood extracted from tissues that have been exposed to varied environmental conditions. Extracted blood containing as little as 0.5 mg. of hemoglobin per milliliter and having 10% carboxyhemoglobin has been analyzed satisfactorily. Samples containing concentrations of less than 10% carboxyhemoglobin show an analytical error of less than 10% of the determined value, and samples containing carboxyhemoglobin concentrations greater than 10% have an analytical error of less than 5%. No interference from either methane, ethane, hydrogen sulfide, acetylene, hydrogen, argon, carbon dioxide, or the products of decomposition was observed. Although the described procedure is used for the determination of carbon monoxide in blood, it will serve to demonstrate an approach that may be employed to liberate other gases.

RESULTS

The effect of decomposition induced by sea water submersion on the quantitative estimation of carbon monoxide in animals without carbon monoxide exposure is illustrated in Table 1. Prior to water exposure, the blood was devoid of any detectable quantities of carbon monoxide in five of the animals. In the remaining two animals there were noted trace quantities of carbon monoxide, i.e., 0.5% and 1.0% carboxyhemoglobin. After submersion in sea water, the final percentage of carboxyhemoglobin in all the animals studied was minimal, ranging from zero to 2.5% carboxyhemoglobin. When possible, confirmatory studies were performed on either liver or the fluid contents from the thoracic cavity. The carboxyhemoglobin findings on either blood

extracted from the liver or the sanguineous fluid recovered from the thoracic cavity were in general agreement with the heart-lung data. No marked increase in the carbon monoxide concentration was noted.

TABLE 1.

PERCENT CARBOXYHEMOGLOBIN IN ANIMALS NOT EXPOSED TO CARBON MONOXIDE

Dog	Prior to submersion	After submersion in sea water (4 days)		
	Blood	Blood extract from heart-lung	Sanguineous fluid from thoracic cavity	Blood extract from liver
1	0.0	2.0	1.0	-
2	1.0	2.5	1.0	-
3	0.0	1.0	-	1.0
4	0.0	2.0	-	0.0
A	0.0	0.0	-	0.0
B	0.0	2.0	-	0.0
C	0.5	1.0	-	0.5

The carbon monoxide level observed in animals containing a relatively low concentration of carbon monoxide in the blood prior to submersion in sea water is shown in Table 2. Exposure to 1% carbon monoxide in air induced blood levels ranging from 4.0% to 12.0% carboxyhemoglobin prior to death. In this group of animals, blood extracted from a mixture of heart and lung tissue proved to be the most suitable specimen for carbon monoxide study. The aqueous extracts of blood were examined for the presence of carbon monoxide as soon as possible after their preparation. In general, a satisfactory correlation was noted in the percent carboxyhemoglobin concentration observed in the blood samples taken prior to death when compared with the aqueous tissue extracts. There was neither a severe loss nor an increase in the percentage of carboxyhemoglobin in these animals following decomposition. Although it was possible to secure sanguineous fluid from the thoracic cavity in only three animals, the carbon monoxide results observed are in general agreement with the presubmersion carbon monoxide blood values. In addition, findings in confirmatory studies performed on blood extracted from liver specimens in three other animals, also are in agreement with those on the control blood.

TABLE 2

PERCENT CARBOXYHEMOGLOBIN IN ANIMALS

EXPOSED TO CARBON MONOXIDE

Dog No.	Prior to submersion	After submersion in sea water (4 days)		
	Blood	Blood extract from heart-lung	Sanguineous fluid from thoracic cavity	Blood extract from liver
5	4.5	5.0	7.5	6.0
6	12.0	10.0	-	-
7	4.0	3.5	-	-
8	10.0	10.5	-	10.0
9	4.5	5.0	-	5.0
10	4.5	6.0	-	7.0
11	6.0	6.0	10.0	-
12	6.0	5.0	5.0	-

In the investigations reported in Tables 1 and 2, rigid measures were employed to prevent any additional postmortem alterations in the tissue specimens following their collection at the experimental site. The samples were maintained in a frozen condition until examined for carbon monoxide and the extracts were analyzed as soon as possible after extraction. Table 3 shows the percent carboxyhemoglobin observed when a combination of heart and lung tissue was examined after being exposed to the air at room temperature. The highest value noted in these representative cases was 35.5% carboxyhemoglobin saturation, as compared to the original 6.0% carboxyhemoglobin value. In this preliminary study no attempt was made to explore the maximum percent carboxyhemoglobin level that might be obtained in these decomposed tissue samples. Treatment of the extracts with $Na_2S_2O_4$ reduced the carboxyhemoglobin figure, in almost all cases, to approximately the level noted in the original determinations.

TABLE 3

COMPARISON OF PERCENT CARBOXYHEMOGLOBIN SATURATION LEVELS

IN FROZEN AND IN THAWED AND REFROZEN TISSUE

Dog	Frozen tissue	Thawed and refrozen tissue	
		Untreated	Treated with sodium hydrosulfite ($Na_2S_2O_4$)
3	1.0	5.0	2.0
4	2.0	9.0	2.0
1	2.0	10.0	2.5
2	2.5	12.5	4.0
7	3.5	10.0	6.0
9	5.0	13.0	5.0
5	5.0	16.0	7.0
8	10.5	22.0	10.0
12	5.0	31.0	8.5
10	6.0	35.5	9.5

DISCUSSION

The interpretation of the significance or reliability of elevated carboxyhemoglobin findings in aircraft-accident victims associated with marked postmortem decomposition has been difficult. In addition to the well-known difficulties related to any toxicologic study involving decomposed specimens, evidence suggesting the in vitro formation of carbon monoxide in blood and blood extracted from tissue has been presented by several investigators (refs. 7, 5, 2). These observations dealing with the formation of carbon monoxide have not been limited to solely hematogenous sources; for example, the phytogenous origin of carbon monoxide has been reported (refs.6, 8). Still another important consideration appears to be the preservation of the specimen following collection and prior to the actual estimation for carbon monoxide.

In the present report, however, no increase was observed in carbon monoxide levels when rigid measures were employed to insure the proper preservation of the samples. No marked increase in the percentage of carboxyhemoglobin was observed in either the control or the carbon monoxide-treated animals. An important finding is the apparent absence of the loss of carbon monoxide with decomposition at low carboxyhemoglobin concentrations, e.g., 4% to 12% carboxyhemoglobin. This is within the range of carbon monoxide results for man, which are of considerable interest to the medical investigators in aircraft accident studies (ref. 3). On the basis of the findings reported here, it appears possible to obtain carbon monoxide values in blood extracted from decomposed postmortem tissue that are similar to the antemortem

levels. Whether a similar agreement in antemortem and postmortem carboxyhemoglobin results would be obtained with higher carboxyhemoglobin concentrations, however, remains to be determined.

The potential difficulty in attempting to interpret carbon monoxide results obtained with specimens that have not been properly preserved is illustrated in this report. In these cases, unreliable carboxyhemoglobin results are possible, as noted by the elevations in percentage carboxyhemoglobin values. Since the carboxyhemoglobin levels are not significantly altered in frozen specimens the extracts of which have been examined immediately after preparation and the analytical method utilized is specific for carbon monoxide, the apparent increase reflected in the analysis appears to be related to alterations initiated during the thawing and/or the preparation of the extracts. The spurious increase in carboxyhemoglobin values obtained in this study may be explained on the basis of: (a) the relative stability of the carboxyhemoglobin molecule in relation to the stability of hemoglobin and the method employed to establish the percentage carboxyhemoglobin saturation value, and (b) the chemical alteration of hemoglobin. Each of these factors will be discussed.

(a) Spurious results attributable to relative stability of carboxyhemoglobin and hemoglobin: The percentage carboxyhemoglobin value is derived by comparing the peak height response of the carbon monoxide content and carbon monoxide capacity of a specimen. Therefore, in the event the hemoglobin in a sample has been altered and this alteration inhibits the capacity of every bit of the hemoglobin to combine with carbon monoxide, the carbon monoxide capacity of the sample would be artificially low, thus indicating a lower concentration of hemoblobin than actually present. In addition, the carboxyhemoglobin complex is relatively stable, and therefore the carbon monoxide content should remain relatively unchanged upon gas-chromatographic analysis. In other words, the rate of carboxyhemoglobin breakdown does not parallel the much more rapid breakdown rate to hemoglobin. Therefore, an increase in the percentage of carboxyhemoglobin saturation will be observed with a reduction in the carbon monoxide capacity of a specimen, while the corresponding carbon monoxide content analysis remains relatively unchanged.

(b) Influence of alterations in hemoglobin: As an aid in evaluating the carbon monoxide results in cases associated with postmortem decomposition, it is important to compare the composition of a fresh blood specimen with the blood available in postmortem cases. In contrast to a fresh blood specimen for carbon monoxide evaluation, where the hemoglobin is entirely in the form of carboxyhemoglobin, oxyhemoglobin, and reduced hemoglobin, the blood extracted from tissue and available for examination in decomposed cases has been subjected to autolysis, hemolysis, and bacterial decomposition. In addition, the original active hemoglobin will be altered by denaturization as well as with formation of sulfhemoglobin and methemoglobin. In drawn blood or laked blood, the spontaneous conversion of hemoglobin to methemoglobin takes place to an appreciable extent (ref. 1). The *in vitro* formation of small amounts of carbon monoxide in whole blood has been correlated with hemoglobin breakdown, and this parallels the formation of methemoglobin (ref. 7).

Therefore, these observations suggest strongly that the elevations in the carboxyhemoglobin saturation values noted are related to the in vitro formation of methemoglobin and the partial inactivation of hemoglobin. Hemoglobin may be inactivated, with respect to its capacity to bind with carbon monoxide, by its conversion to methemoglobin. Since it has been observed, as cited by Bodansky (ref. 1), that methemoglobin lacks the capacity to combine with carbon monoxide, the conversion of hemoglobin to methemoglobin represents another means whereby the carbon monoxide capacity of a sample may be reduced, indicating a lower level of hemoglobin than actually present. The capacity of methemoglobin to increase the percentage carboxyhemoglobin value is evident in this study, in which spurious elevations as high as 35% carboxyhemoglobin saturation were attained. With the use of sodium hydrosulfite, which reconverts methemoglobin to reduced hemoglobin, it was possible to reduce elevations in the order of 31.0% and 35.5% carboxyhemoglobin saturation to 8.5% and 9.5% carboxyhemoglobin saturation, respectively. The action of sodium hydrosulfite may be attributed to the capacity of this chemical agent to reduce the ferric ion of methemoglobin to ferrous ion. Once this has occurred, all the available hemoglobin is again in a form capable of combining with carbon monoxide. Thus an accurate level of hemoglobin is obtained, as reflected in the determination of carbon monoxide capacity.

SUMMARY AND CONCLUSIONS

The evidence presented indicates that the percentage carboxyhemoglogin saturation is not markedly altered during postmortem decomposition when (1) the specimens are properly preserved (i.e., upon collection, the specimens are rapidly frozen and maintained in a frozen state until examined for carbon monoxide), and (2) the blood extracted from tissue is examined as soon as possible for the presence of carbon monoxide. Under the conditions studied, it appears possible to obtain carbon monoxide values, utilizing blood extracted from tissue, in the presence of postmortem decomposition, that are similar to the antemortem levels. For example, within the 4% to 12% carboxyhemoglobin-saturation range covered in this study, there was neither a marked increase nor decrease in final percentage of carboxyhemoglobin following decomposition. Factors are discussed that should be considered in the interpretation of carbon monoxide findings in cases associated with marked postmortem decomposition.

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Aerospace Medical Division, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio. Rpt. No. AMRL-TDR-63-69. DETERMINATION OF CARBOXYHEMOGLOBIN IN DECOMPOSED BODIES. Final report, Jul 63, iii + 15 pp. incl. illus., tables, 8 refs. Unclassified report

This study is concerned with the interpretation and the significance of carbon monoxide findings in cases associated with decomposition. The evidence presented indicates that the percentage of carboxyhemoglobin saturation value is not markedly altered during postmortem decomposition when: 1) the specimens are properly preserved (i.e., upon collection the specimens are rapidly frozen and maintained in a frozen state until examined for carbon monoxide), and

(over)

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1. Hemoglobin
2. Carbon Compounds and Monoxides
3. Toxicity
4. Laboratory Animals
5. Decomposition

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2) the blood extracted from tissue is examined as soon as possible for the presence of carbon monoxide. Under the conditions studied, it appears possible to obtain carbon monoxide values, utilizing blood extracted from tissue, in the presence of postmortem decomposition that are similar to the antemortem levels. For example, within the 4% to 12% carboxyhemoglobin-saturation range covered in this study, there was neither a marked increase nor decrease in final percentage of carboxyhemoglobin following decomposition. Nevertheless, unreliable carboxyhemoglobin saturation values may occur, and the possible contributory factors responsible for a spurious increase in carboxyhemoglobin values are discussed along with corrective measures. A gas-solid chromatographic procedure for determining carbon monoxide using blood or tissue is presented.

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VI. In DDC collection
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